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Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Developmental biology and prey preference of *Diomus notescens* Blackburn (Coleoptera: Coccinellidae): A predator of *Aphis gossypii* Glover (Hemiptera: Aphididae)



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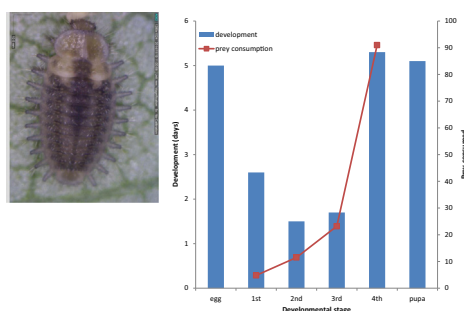
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HIGHLIGHTS

- Development and reproduction of *D. notescens* preying on *A. gossypii* was recorded.
- The intrinsic rate of increase of *D. notescens* was estimated to be 0.14 under laboratory conditions.
- The thermal requirements of *D. notescens* were calculated based on development at four temperatures.
- *D. notescens* has a strong preference for *A. gossypii* compared to *B. tabaci*.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 21 July 2015

Revised 4 February 2016

Accepted 9 February 2016

Available online 13 February 2016

Keywords:

Biological control

Cotton aphid

Development

Predation

Degree-days

Minute two-spotted ladybeetle

Silverleaf whitefly

ABSTRACT

The minute two-spotted ladybeetle, *Diomus notescens* Blackburn is a common predator of aphids and other pests in Australian agricultural crops, however little is known about the biology of *D. notescens*. The aim of this study was to provide information on the life cycle of this predator and improve our understanding of its biological control potential, particularly against one of the major pests of cotton, *Aphis gossypii* Glover. In laboratory experiments, juvenile development, prey consumption, as well as adult lifespan and fecundity were studied. Results from this study revealed that *D. notescens* could successfully complete development on *A. gossypii*, which at 25 °C required 21 days and during this period they each consume 129 ± 5.2 aphids. At 25 °C adult lifespan was 77 ± 9.6 days, with a mean daily prey consumption of 28 ± 1.8 aphids and a mean daily fecundity of 8 ± 0.5 eggs. Net reproductive rate was estimated as 187 ± 25.1 females and the intrinsic rate of increase was estimated as 0.14. Juvenile development was recorded at four constant temperatures (15, 21, 26 and 27 °C) and using a linear model, the lower threshold for *D. notescens* development was estimated to be 10 ± 0.6 °C with 285 ± 4.7 degree days required to complete development. A prey choice experiment studying predation rates revealed a strong preference for *A. gossypii* nymphs compared to *Bemisia tabaci* Gennadius eggs.

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1. Introduction

The cotton or melon aphid, *Aphis gossypii* Glover is a major worldwide pest of cotton and horticultural crops (Blackman and Eastop, 2000). Current management practices claim to adopt 'integrated pest management' (IPM), but this often translates to pest

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sampling plans to direct the strategic application of selective insecticides, with no real integration of natural enemies into the decision-making process (Zalucki et al., 2009; Zalucki et al., 2015). As a result management relies heavily on insecticidal control, in particular neonicotinoids (Herron and Wilson, 2011). Such an approach is not likely to be sustainable due to the propensity of *A. gossypii* to develop resistance to insecticides (Herron et al., 2001; Herron and Wilson, 2011) and the future prospect of the deregistration or restrictions being placed on the usage of insecticides in response to environmental and human health concerns (Hillocks, 2012). As a result a greater emphasis on the use of other IPM options needs to be considered, including the use of biological control. For biological control to be effective we need to establish a clear understanding of the impact natural enemies can have on pest species (Furlong and Zalucki, 2010).

Over the past fifteen years, pest management of cotton in Australia has undergone a transition from frequent use of broad spectrum insecticides to control *Helicoverpa* spp. and other (mostly hemipteran) pests, to wide spread adoption of genetically modified cotton that produces toxins that kill the larvae of *Helicoverpa* species, which has resulted in a large reduction in insecticide application (Mensah et al., 2015). While other pests are still controlled using insecticides there has been a large extension effort in cotton to adopt IPM (Pyke and McIntyre, 2007). With these changes in pest management there is now an opportunity to improve the role of predators in the biological control of pests.

While simply reducing the use of insecticides may appear to increase biological control through increasing predator biodiversity in agricultural ecosystems, such biodiversity alone may not be enough to increase pest suppression if key predator species are absent or at low densities (Straub and Snyder, 2006). To effectively use predators in biological control, an understanding of their basic biology (development, suitability of the pest as prey, habitats, crop occupancy) is needed so that the impact of the predator on pest populations might be modelled and used to develop predator to pest ratios that can be used by agronomist – pest management advisors (Furlong and Zalucki, 2010; Johnson et al., 2000).

Coccinellids including those in the genus *Diomus* Mulsant can contribute to the biological control of pests such as aphids and mealybugs in agricultural and horticultural crops, including cotton, sorghum, sugarcane and citrus (Akbar et al., 2009; Auad et al., 2013; Chong et al., 2005; Conway and Kring, 2010; Hentz and Nuessly, 2002; Meyerdirk, 1983; Tiffet et al., 2006). *Diomus notescens* Blackburn is a common and widely distributed coccinellid in Australia (Pang and Slipinski, 2009) (also found in New Zealand) and has been recorded in many agricultural crops (Johnson et al., 2000). *Diomus notescens* is recorded as a predator of aphids, including *A. gossypii* (Bishop and Blood, 1978; Stanley, 1997) and other small prey like spider mites in Australian cotton crops (Williams et al., 2011). Little else is known about the biology *D. notescens* and its role in biological control in cotton is unknown.

The development of *D. notescens* in response to temperature, prey consumption by the larval and adult stage, lifespan and fecundity of adults, and prey preference of adults was investigated in a series of laboratory experiments. The intrinsic rate of increase of *D. notescens* was estimated to benchmark its biological control potential and to draw some conclusions about its effectiveness as a predator of *A. gossypii*.

2. Methods

2.1. Insects and plants

Diomus notescens were collected from barley plants hosting *Rhopalosiphum padi* (L.) near Toowoomba (−27.5351, 151.9302),

Queensland, Australia in 2013. *Aphis gossypii* and Silverleaf whitefly, *Bemisia tabaci* Gennadius were collected from cotton growing near Emerald (−23.5234, 148.1553), Queensland, Australia in 2013. Stock cultures of all insects were kept in a glasshouse in rearing cages containing potted cotton plants (variety Sicot 71RRF); *A. gossypii* and *B. tabaci* on their own and *D. notescens* with *A. gossypii* as prey.

2.2. Development and prey consumption

Adult *D. notescens* were collected from the rearing cages, transferred into plastic containers (16 × 12 × 6 cm) lined with damp paper towel and kept in a constant temperature room at 25 ± 3 °C, 60 ± 12% RH and 15:10 L:D. Leaves infested with *A. gossypii* were added as food and the paper towel was checked daily for eggs. Sections containing eggs were cut out and stored in Petri dishes (8 cm diameter) without vents. To keep the leaf material fresh, a moist filter paper (Whatman® 1) was used to line the bottom of the dish. Eggs in the Petri dishes were checked regularly for larval emergence.

Forty newly emerged larvae (<12 h old) were carefully moved into individual experimental arenas, consisting of a cotton leaf with 30 2nd instar *A. gossypii* nymphs kept in an unvented Petri dish (8 cm diameter). To keep the leaf fresh, a moist filter paper (Whatman® 1) was used to line the bottom of the dish. Petri dishes were kept in a constant temperature room at 25 ± 3 °C, 60 ± 12% RH and 15:10 L:D. The developmental instar of each ladybeetle larva and number of aphids consumed was recorded daily, and aphids were added once a day to replace those eaten. Every second day, leaves and all aphids were replaced to keep the aphid development stages within a range of 2nd to 4th instar.

Prey consumption of adult male (n = 9) and female (n = 11) *D. notescens* were measured individually using the same experimental arena described above; however the number of aphids was increased to 75 nymphs (2nd or 3rd instar). Adults typically consumed the entire aphid, leaving little evidence of predation behind; as a result aphid consumption was based on number of aphids missing. Five controls were set up to estimate aphid mortality in the experimental arena in the absence of the ladybeetle predator. For each replicate the number of aphids missing and was recorded daily and for the female ladybeetles number of eggs laid was recorded each day. Prey consumption was recorded for 54 days; fecundity and lifespan were recorded till the last individual died. Beetles were kept together for several hours after emergence to mate before being used in the experiment and beetles were paired together again at 40 days to mate.

Data was collected on duration of egg, larval and pupal development at 25 °C, number of aphids consumed in each instar and as adults, as well as lifespan and fecundity of adults.

2.3. Effect of temperature on development

To estimate the temperature developmental threshold of *D. notescens*, juvenile stages were reared at four temperatures: 15, 21, 26 and 27 °C. Adults were kept in plastic containers (as described above) to obtain eggs. For each treatment, approximately 50 eggs (<12 h old) were placed into an unvented Petri dish (8 cm diameter) lined with moist filter paper and incubated at the treatment temperature. As larvae hatched they were moved to a Petri dish, with a moist filter paper and a cotton leaf infested with *A. gossypii*. Stage of development and survival of immature ladybeetles was recorded daily. The temperature and humidity inside each incubator was recorded every hour using a data logger (Tinytag®).

2.4. Prey preference

Prey choice of adult *D. notescens* in response to different life stages of *B. tabaci* was investigated in two experiments. In the first, adult beetles were individually ($n = 5$) released into an experimental arena (Petri dish 8 cm diameter) containing a cotton leaf with equal numbers ($n = 20$) of whitefly eggs, 2nd instar nymphs and adults. Predation by the beetles was observed for 30 min and then they were left 24 h. At this time total number of prey consumed was recorded. In the second experiment, prey choice of 10 beetles was assessed by placing them individually in experimental arenas with 10 1st instar and 10 4th instar *B. tabaci* nymphs. The number of prey eaten was recorded after 24 h.

Adult *D. notescens* prey choice in response to the presence of *B. tabaci* and *A. gossypii* was studied by placing them into an experimental arena (Petri dish 8 cm diameter) with 80 prey in the following ratios of *B. tabaci* to *A. gossypii*; 0:80, 20:60, 40:40, 60:20 and 80:0. *Bemisia tabaci* were offered as eggs, based on earlier tests of life stage preference, and *A. gossypii* as 1st to 2nd instar nymphs. Treatment leaves were set up by confining female *B. tabaci* with a clip cage (35 mm diameter by 10 mm high) to a cotton leaf for several hours to lay eggs. Location and number of eggs were marked and any excess eggs were removed. *Aphis gossypii* were added to the leaf with a fine brush and left overnight to settle. Prior to adding the predator, prey numbers were checked and additional aphids added if needed. For each treatment (prey ratio) 10 replicates were conducted, with one adult ladybeetle being individually tested per replicate. After five hours the number of remaining *A. gossypii* and *B. tabaci* were counted for each replicate. For each treatment two controls were setup where a ladybeetle was not added so mortality could be corrected based on numbers left in the control after 5 h.

2.5. Data analysis

Adult aphid consumption data was corrected for control mortality using the Henderson-Tilton formula (Henderson and Tilton, 1955), and then a one way analysis of variance (ANOVA) was completed to test for differences in female and male daily aphid consumption.

Lifespan and egg count data was used to produce fecundity life tables. Age-specific survival (l_x), fertility (m_x), net reproductive rate (R_0), mean generation time (T) were calculated and used to estimate the intrinsic rate of natural increase (r_m) using the iterative bisection method from the Euler-Lotka formula (Birch, 1948). The Jackknife technique (Sokal and Rohlf, 1995) was used to estimate the mean and SE of r_m (Yu et al., 2013).

$$\sum_{x=0}^{\infty} e^{-r_m(x+1)} l_x m_x = 1$$

Predator preference was calculated by applying the following formula to the data, which is a specific case of Manly's preference index (Manly, 1974):

$$\beta_1 = \frac{\ln\left(\frac{e_1}{A_1}\right)}{\ln\left(\frac{e_1}{A_1}\right) + \ln\left(\frac{e_2}{A_2}\right)}$$

where the index β_1 is the predators' preference for prey type 1, e_1 is the number of surviving prey belonging to prey type 1, A_1 is the number of prey type 1 offered, e_2 is the number of prey type 2 remaining and A_2 is the number of prey type 2 offered. The value of β_1 will fall between 0 and 1. An index close to 1 indicates preference for prey type 1 and an index close to 0 indicates preference for prey type 2. A value close to 0.5 indicates the predator selects prey randomly, showing no preference. The preference of *D. notescens* for

one type of prey over the other was tested by comparing the β_1 values of *B. tabaci* eggs and *A. gossypii* nymphs using an ANOVA followed by least significant difference test. Prey switching in *D. notescens* was tested using a Student's *t*-test that compared the estimated β_1 values with expected values (Jaworski et al., 2013).

Differences in development time of each immature stage at each temperature were tested for significance by ANOVA using a general linear model. Means were separated by least significant difference test after a significant *F*-test at $p = 0.05$. The relationship between temperature (T) and developmental rate (Dr , the reciprocal of developmental time in days) was determined by the linear model: $Dr(T) = aT + b$, where a and b are constants that were estimated by linear regression. The lower thermal threshold for development (t) and thermal constant (K number of degree-days above t to complete immature development) as well as their respective standard errors were calculated as described by Campbell et al. (1974).

All analysis was completed in Genstat 16th edition.

3. Results

3.1. Development and prey consumption

At 25 °C, eggs of *D. notescens* took an average (\pm se) of 5.0 ± 0.1 days to hatch, larval development took 11.1 ± 0.4 d, and pupal development took 5.3 ± 0.3 d (Table 1). To complete larval development, *D. notescens* consumed a mean of 129 ± 5.2 aphids (Table 1). Consumption of aphids steadily increased with each successive instar. An approximate 24 h pre-pupal period at the end of the 4th instar was spent searching for a location for pupation, during this period aphid consumption ceased.

During the 54 days of observation, the mean daily aphid consumption of adult *D. notescens* ($n = 20$) was 28 ± 1.8 aphids. Female *D. notescens* consumed a daily mean of 34 ± 1.7 aphids, which was significantly ($F_{1,19} = 34.11$, $P = <0.001$) more than males, which consumed 21.0 ± 1.2 aphids per day.

Average lifespan ($n = 17$) of *D. notescens* was 77 ± 9.6 d (range 7–145), with average male lifespan 78.5 ± 26.7 d and females 74.7 ± 11.3 d. After eclosion there was about a 4–5 day pre-oviposition period, average fecundity ($n = 9$) over the life of *D. notescens* was 581 ± 89 eggs with a daily average of 8 ± 0.5 eggs laid. Female survival rate (l_x) and daily fecundity rate (m_x) were calculated (Fig. 1). From those calculations a net reproductive rate (R_0) was estimated at 187.3 ± 25.1 assuming a 1:1 sex ratio. The intrinsic rate of increase (r_m) was estimated as 0.14 ± 0.002 females/female/day.

3.2. Effect of temperature on development

Overall development of immature *D. notescens* decreased as temperature increased. At each temperature tested, development time of egg and pupal stages decreased significantly with increasing temperature (Table 2). The estimated lower developmental

Table 1

Mean development (d) and number of aphids \pm SE eaten at 25 °C for each instar of *Diomus notescens*.

Stage	Development (d)	No. of aphids eaten
Egg	5.0 ± 0.1	
1st instar	2.6 ± 0.2	4.8 ± 1.4
2nd instar	1.5 ± 0.2	11.5 ± 1.8
3rd instar	1.7 ± 0.14	23.2 ± 1.6
4th instar	5.3 ± 0.3	91.0 ± 5.3
Total larval	11.1 ± 0.4	129.0 ± 5.2
Pupa	5.3 ± 0.3	

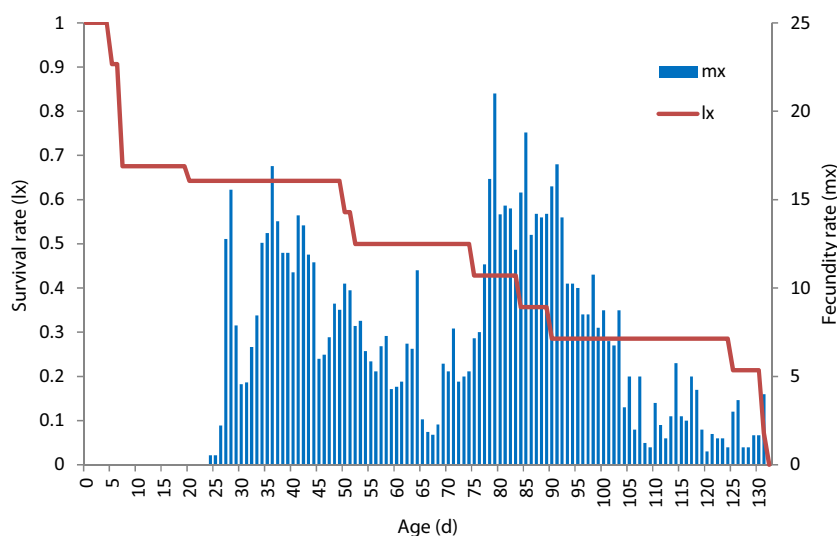


Fig. 1. Age specific survival (lx = proportion alive) and fecundity (mx = number of eggs/female) of *Diomus notescens* feeding on *A. gossypii* at 25 °C.

Table 2

Effect of temperature on development in days (means \pm se) of *Diomus notescens*.

Stage	15 °C	21 °C	26 °C	27 °C
Egg	14.5 \pm 0.11 (67) a	6.7 \pm 0.11 (57) b	5.1 \pm 0.04 (39) c	4.4 \pm 0.16 (27) d
1st instar	5.7 \pm 0.18 (39) a	3.5 \pm 0.17 (39) b	2.1 \pm 0.07 (24) c	1.7 \pm 0.09 (25) c
2nd instar	3.8 \pm 0.16 (38) a	2.2 \pm 0.09 (38) b	1.4 \pm 0.16 (21) c	0.92 \pm 0.07 (25) d
3rd instar	4.3 \pm 0.15 (36) a	1.9 \pm 0.10 (35) b	1.1 \pm 0.07 (20) c	1.35 \pm 0.13 (23) c
4th instar	7.2 \pm 0.29 (35) a	4.8 \pm 0.18 (32) b	2.3 \pm 0.19 (20) c	2.5 \pm 0.20 (23) c
Pre pupa	5.5 \pm 0.34 (33) a	2.1 \pm 0.13 (32) b	1.9 \pm 0.18 (20) b	1.61 \pm 0.21 (23) b
Total larval	26.2 \pm 0.47 (33) a	14.7 \pm 0.34 (32) b	8.85 \pm 0.22 (20) c	8.0 \pm 0.33 (23) c
Pupa	15.3 \pm 0.30 (31) a	6.1 \pm 0.16 (30) b	4.8 \pm 0.16 (20) c	3.91 \pm 0.19 (22) d
Total	55.2 \pm 0.61 (31) a	27.0 \pm 0.35 (30) b	18.7 \pm 0.33 (20) c	16.0 \pm 0.40 (22) d

Means in each row followed by the same letter are not significantly different in ANOVA, using LSD tests at $P = 0.05$.

Values in parentheses are number of ladybeetles tested.

threshold (t) is 10.1 ± 0.6 °C (Fig. 2). It was estimated that the thermal requirements (K) for total immature development is 285 ± 4.7 degree days (DD). Mortality during development was recorded and calculated as a percentage (Table 3). At 27 °C, egg mortality was difficult to assess as low humidity dried out many leaves, causing egg desiccation. This mortality wasn't included in our estimate due to it being an artefact of the incubator. If it was included mortality of eggs at 27 °C would be 71%.

3.3. Prey preference

Diomus notescens preferred *B. tabaci* eggs compared to 2nd instar nymphs or adults; after 24 h a mean of 19 out of 21 available eggs were eaten compared to 2 out of 21 2nd instar nymphs and 0 out of 21 adults. In the second experiment there was no difference in the predation rates of 1st instar (mean of 6.6 out of 10) and 4th instar *B. tabaci* nymphs (mean of 6.1 out of 10).

During the preference study investigating species choice, *D. notescens* rarely fed on *B. tabaci* eggs when *A. gossypii* nymphs were present (Table 4). The Manly index of preference for *A. gossypii* was above 0.9 at each ratio indicating *D. notescens* will more frequently choose *A. gossypii* nymphs as prey items compared to *B. tabaci* eggs. At each ratio of *B. tabaci* to *A. gossypii*, preference for *A. gossypii* was significant, 20:60 ($F_{1,19} = 1398.57$, $P < 0.001$), 40:40 ($F_{1,19} = 3498.68$, $P < 0.001$) and 60:20 ($F_{1,19} = 2.6E + 05$, $P < 0.001$).

Prey switching in response to the most abundant prey did not occur; instead *D. notescens* preyed on *A. gossypii* nymphs regardless

of prey ratio. At each prey complex ratio, the β value was significantly different from the expected β value; at the 20:60 ratio of *B. tabaci* to *A. gossypii* ($t = -26.44$, $df = 9$, $P < 0.001$), 40:40 ratio ($t = -41.83$, $df = 9$, $P < 0.001$) and at 60:20 ($t = -362.17$, $df = 9$, $P < 0.001$).

4. Discussion

Diomus notescens larvae readily fed and successfully developed while feeding on *A. gossypii* nymphs. Larvae tended to attack the aphid nymphs by biting their legs (Hentz and Nuessly, 2002) and then proceeded to feed on the internal contents of the aphid nymphs and leave behind a collapsed exoskeleton (Hentz and Nuessly, 2002), which provided evidence of predation. First instar larvae of the coccinellid were fragile; the highest mortality was observed in the first instar. Many larvae died from starvation, as a result of failing to successfully prey on an aphid within the first 24–48 h after emergence. While a low aphid density can contribute to increased first instar mortality (Hodek, 1996), in *D. notescens* first instars often struggled to successfully attack 2nd instar aphid nymphs. Mortality of first instars was observed to be much lower in situations where first instar larvae could prey on an aphid already being attacked by another coccinellid. Within instars there was limited evidence of cannibalism, but where mixed instars were present, cannibalism was observed with the older instars attacking the younger instars. Larval prey consumption steadily increased with each successive instar, with the 4th instar eating the largest proportion of the total aphids consumed. Towards the

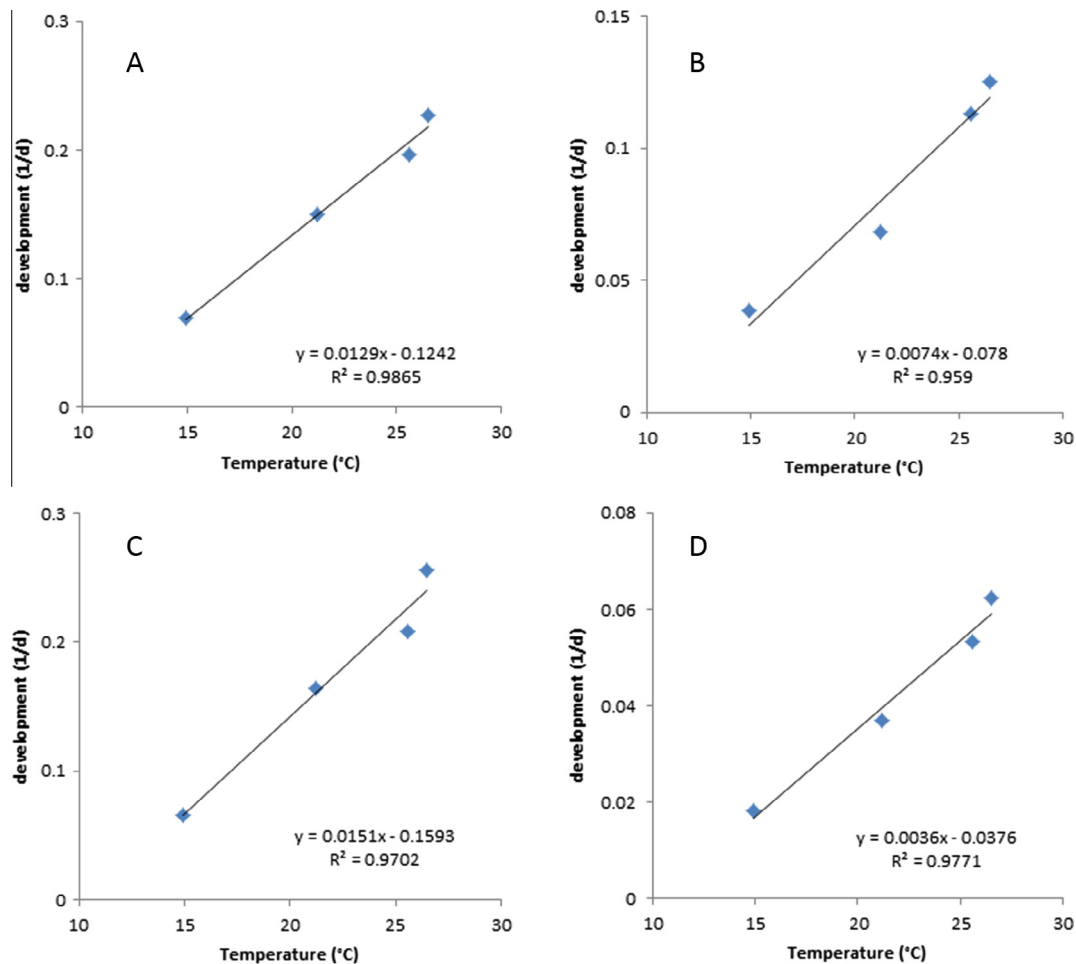


Fig. 2. Observed developmental rate of *Diomus notescens* during (A) egg, (B) larval, (C) pupal and (D) total immature development, lines fit to these data by linear relationship.

Table 3

Effect of temperature on mortality (mean % \pm se) of *Diomus notescens*.

Mortality	15 °C	21 °C	26 °C	27 °C
Egg	9.0 \pm 5.36	17.0 \pm 14.80	11.6 \pm 4.95	4.0 \pm 0.0
1st instar	25.7 \pm 8.93	15.45 \pm 10.12	13.5 \pm 5.21	9.6 \pm 5.78
2nd instar	0.0 \pm 0.0	2.5 \pm 2.50	4.2 \pm 4.17	8.3 \pm 8.33
3rd instar	1.6 \pm 1.59	4.9 \pm 3.05	0.0 \pm 0.0	0.0 \pm 0.0
4th instar	1.8 \pm 1.79	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
Pre pupa	5.4 \pm 3.72	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
Pupa	6.5 \pm 4.80	5.56 \pm 3.51	0.0 \pm 0.0	4.8 \pm 4.76
Total	44.8 \pm 14.63	35.2 \pm 7.80	26.4 \pm 6.02	26.8 \pm 11.20

Table 4

Mean number of prey eaten (\pm SE) by adult *Diomus notescens* on nymphs of *Aphis gossypii* and eggs of *Bemisia tabaci* when offered at different ratios, and the corresponding preference indices (β).

Prey ratio (whitefly: cotton aphid)	Aphid mortality	Whitefly mortality	Preference index for cotton aphid (β)
0:80	23.1 \pm 4.1		
20:60	20.6 \pm 1.8	0.4 \pm 0.2	0.96
40:40	19.0 \pm 1.2	0.4 \pm 0.2	0.98
60:20	11.5 \pm 1.2	0.1 \pm 0.1	0.99
80:0		14.4 \pm 7.8	

end of the 4th instar, feeding stops and individuals enter a prepupal stage where they fix themselves to a leaf or other nearby substrate and pupate; during the experiments they often pupated on the filter paper, while in the rearing cages they often pupated on

the abaxial side of the leaf or in concealed location like the underside of dead leaves on the soil surface.

At temperature ranging from 25 to 27 °C, the time required for *D. notescens* to complete development fell within the range reported for other *Diomus* species. *Diomus flavifrons* (Blackburn) required 29 days to complete development at 24 °C (Meyerdirk, 1983), *D. terminatus* (Say) completes development in 15 days at 27.5 °C (Hentz and Nuessly, 2002). Two studies have investigated the relationship between temperature and development in *Diomus* species. When fed on *Sipha flava* (Forbes), *Diomus seminulus* (Mulsant) requires 61, 31, 22 and 18.5 days at 16, 20, 24 and 28 °C (Aquad et al., 2013). Development of *Diomus austrinus* Gordon feeding on *Planococcus citri* (Risso) and *Phenacoccus madeirensis* Green has been studied at 15, 20, 30 and 35 °C. Successful development was possible in the range of 20–30 °C and required 45, 25.6 and 16.7 d at 20, 25 and 30 °C when fed *P. citri* (Chong et al., 2005).

At 10.1 °C, the minimum threshold temperature for *D. notescens* is about 4 °C lower than that estimated for *D. austrinus* and applying the theoretical prediction (Dixon et al., 2009) that there is a 20 °C window between t_{min} and t_{max} it would predict an upper thermal tolerance of 30 °C for *D. notescens*, while for *D. austrinus* it would be 34 °C. In the study of *D. austrinus*, egg and larval development through to 4th instars was recorded at 35 °C, but larvae did not complete the prepupal stage (Chong et al., 2005). The thermal tolerances of both species correlate well with their geographic ranges; *D. austrinus* appears to be limited to Southern Florida (Chong et al., 2005) which has a tropical climate, while *D. notescens* has a wider distribution from tropical to temperate climatic zones

in Australia (Pang and Slipinski, 2009). The thermal requirements to complete development of *D. australis* (240.4–261.8 DD) and *D. notescens* (285 DD) are similar and slight differences are likely due to different diets (as can be seen in *D. australis*) and different experimental conditions, rather than differences in metabolic processes in the two species.

The thermal requirements of *D. notescens* are very different to those of *A. gossypii* which has a threshold temperature of 6.3 °C (Parajulee, 2007) to 7.1 ± 2.5 °C (Xia et al., 1999) and requires 99.4 ± 14.2 DD for development (Xia et al., 1999); *A. gossypii* can develop faster and at lower temperatures compared to *D. notescens*. With *A. gossypii*, the shortest development time occurs between 27.5 and 30 °C (Akey and Butler, 1989; Kersting et al., 1999; Xia et al., 1999), for *D. notescens* the optimal temperature wasn't determined in this study. High egg mortality was observed at 27 °C, due largely to the low humidity in the incubator at this temperature. Low egg viability was reported in *Diomus seminulus* (Mulsant) at 28 °C, but immature development was still possible (Aquad et al., 2013). Further studies are needed investigating the effects of humidity and temperatures of 30 °C and above on *D. notescens* development.

There are no published studies that detail the lifespan and rates of prey consumption of adult *Diomus* species. So it is difficult to compare our study with others due to variation in number of prey provided and the incomplete nature of most observations. Even in our study we stopped recording prey consumption at a fixed point rather than continue to the death of all study insects. That said the lifespan of *D. notescens* would appear similar to *D. terminatus* which has been reported to live up to maximum of 143 days when fed *A. gossypii* and 75 days on *Myzus persicae* (Sulzer) (Hallborg, 2003). While (Hentz and Nuessly, 2002) reported that several beetles survived up to 50 days on *S. flava*. Daily prey consumption rates of *D. terminatus* range from 8.7 on *M. persicae*, 13.5 on *A. gossypii* to 19 on *Melanaphis sacchari* (Zehntner) (Akbar et al., 2009; Hallborg, 2003).

Fecundity of *D. notescens* observed in this study was much greater than the fecundity of other *Diomus* species reported in the literature. The lifetime fecundity of *D. terminatus* ranges from 37 (when feeding on *M. persicae*), 42 (*S. flava*) to 86 (*A. gossypii*) (Hall, 2001; Hallborg, 2003). While the fecundity of *D. seminulus* when fed *S. flava* was 71 eggs per female over 150 days (Aquad et al., 2013) and the fecundity of *D. flavifrons* feeding on *P. citri* is 147 eggs per female (Meyerdirk, 1983). Hallborg (2003) found that *D. terminatus* fed on *Rhopalosiphum maidis* (Fitch) could lay up to 14 eggs per day, but lifetime fecundity was not determined, so it's difficult to compare with our results. Its likely differences in the nutritional quality and quantity of aphids provided explains the large variation in fecundities reported (Hallborg, 2003) assuming *Diomus* species all have a similar number of ovarioles (Honek, 1996). Further, the suitability of the experimental arenas for egg lay could potentially impact on fecundity (Hallborg, 2003; Honek, 1996) and the availability of males in the arena if continued mating is required to maximise fecundity.

In our experiment, egg production continued for the duration of adult life, but egg production had decreased or ceased for some individuals at day 40 when we paired the beetles together for mating again. With the exception of one individual, all the females that had stopped laying recommenced, suggesting more than one mating is required during the lifespan to maximise fecundity.

The estimated intrinsic rate of increase for *D. notescens* was 0.14, which is lower than 0.37 to 0.38 which is the estimate for *A. gossypii* (Parajulee, 2007; Xia et al., 1999) indicating the predator population grows slower in an unlimited environment compared to *A. gossypii*. This is not a great surprise as *A. gossypii*, like many other aphids, has evolved several life traits that facilitate rapid population growth including, an anholocyclic life cycle and parthenogenic reproduction. Comparing the intrinsic rate of increase of two insects with such different reproductive strategies may be of

little value, but it may be useful to compare and rank the potential biological control effectiveness of different predators. However one should be careful arriving at conclusions as intrinsic rate of increase represents potential growth and the conditions experienced in the laboratory (plentiful prey, no adverse weather conditions, no competition for prey and no natural enemy or disease impacts) are unlikely to be experienced in field situations. How resilient different predators are to these conditions may be just as important as their reproductive capacity.

The combination of relatively low consumption rate of adults and the lower rate of population increase suggests that the impact of *D. notescens* on *A. gossypii* would be limited, particularly during the rapid growth phase that can occur in aphids when environmental conditions favour them. The environmental conditions that favour *A. gossypii* are quite specific, and changes in day length, temperature and plant nitrogen levels can result in polymorphisms, such as yellow dwarf form that has an intrinsic rate of increase of around 0.2 (Rosenheim et al., 1994), which is much lower than the values estimated by Xia et al. (1999) and Parajulee (2007). So during summer, when yellow to green forms of *A. gossypii* make up a large proportion of aphid populations, the impact of *D. notescens* may be greater than during spring and autumn when the darker morph of *A. gossypii* is more prevalent (Rosenheim et al., 1994).

While *A. gossypii* populations can grow rapidly at a local level, i.e. a patch within a field; to become a pest they need to colonise a larger area of the field. This is where *D. notescens* may have the most impact, consuming small establishing colonies of aphids either early in the season as *A. gossypii* first arrives or later as established patches start to produce alates and spread further into a field. This assumes there is other prey or sources of food available that can sustain *D. notescens* in the absence of *A. gossypii*.

Diomus notescens will feed on *B. tabaci* eggs and nymphs, but fed more frequently on eggs. When both *A. gossypii* nymphs and *B. tabaci* eggs were present the ladybeetle fed on both prey, but had a strong preference for *A. gossypii* nymphs. The ratio of one prey item to the other had no effect on the prey choice of *D. notescens* indicating they did not switch to feeding on the most abundant prey available.

Like *D. notescens* some species do not change prey choice in response to changing density. The green lacewing *Chrysoperla carnea* (Stephens) for example shows a preference regardless of prey ratio for the aphid *Nasonovia ribisnigri* (Mosley) when offered with *Frankliniella occidentalis* (Pergande) on lettuce (Shrestha and Enkegaard, 2013). Likewise the predatory bugs, *Anthocoris nemorum* (L.) and *Anthocoris nemoralis* (Fabricius) both show a preference for *M. persicae* when it was offered together at equal densities with either *Aulacorthum solani* (Kaltenbach), *Macrosiphum euphorbiae* (Thomas), *A. gossypii* and *Aphis fabae* Scopoli (Meyling et al., 2003).

Switching in response to changing prey densities does occur in some species, for example the adults of the generalist predator *Macrolophus pygmaeus* Rambur will switch to the most abundant prey when offered both *B. tabaci* and the alternative prey *Tuta absoluta* (Meyrick) (Jaworski et al., 2013). In another predatory mirid, *Macrolophus caliginosus* Wagner prey preference for *Trialeurodes vaporariorum* Westwood eggs increases with increasing proportions of this prey type, when present with *Tetranychus urticae* Koch eggs (Enkegaard et al., 2001).

In the absence of *A. gossypii*, the whitefly (*B. tabaci*) eggs were readily found and consumed by *D. notescens*. This suggests factors other than prey size may contribute to the preference for *A. gossypii* observed in this experiment. Prey preference is largely due to trade-offs between various mechanisms, including the ease of attacking the prey and their nutritional value (Eubanks and Denno, 2000; Jaworski et al., 2013). The ease at which prey are attacked is due to various factors, including capacity to detect prey, access to the prey, defence responses of the prey against predation,

and the capacity of the predator to effectively feed on the prey (Jaworski et al., 2013). Both *B. tabaci* eggs and *A. gossypii* nymphs appeared to be easy for *D. notescens* to find and prey on. Handling time of *B. tabaci* eggs was quicker than *A. gossypii* nymphs (JH unpublished observations). Lower nutritional value and quantity of nutrition extracted per feeding event of *B. tabaci* eggs compared to aphids is a likely explanation for the prey preference observed in our experiment. In a study where reproductively active *D. notescens* were added to a cage with all life stages of *B. tabaci*, no reproduction of the ladybeetle was observed a over period of one month which indicates they may not be nutritionally suitable (unpublished data). This observation along with the studies above would indicate that *A. gossypii* can be considered an essential food, while *B. tabaci* is best categorised as an alternative food, which serves only as a source of energy enabling survival of adults in the absence of other more suitable prey (Hodek, 1996). Whether the larvae of *D. notescens* share the prey choice preferences of the adults wasn't determined in our experiments, and would be worth investigating further.

Our study suggests that the contribution of *D. notescens* to pest management of *A. gossypii* appears restricted; their rates of predation and reproduction are not sufficient to control established aphid colonies. Where they are likely to provide greater biological control is in preying on aphids as they establish colonies, either early in the season or through the season as aphids move from established patches to colonise the surrounding field. Their contribution in these roles relies on other prey being sufficient to maintain *D. notescens* and encourage reproduction. *Bemisia tabaci* meets the requirements of an alternative prey, but is not an essential prey. Other prey that might fill the role of essential prey like thrips or mites should be considered in further prey studies of *D. notescens*.

Acknowledgments

We thank Paul Grundy, Tonia Grundy and Janese Lloyd for advice and helpful comments on the manuscript. We are grateful for the funding and support provided by the Cotton Research and Development Corporation that made this research possible.

References

- Akbar, W., Carlton, C., Reagan, T.E., 2009. Life cycle and larval morphology of *Diomus terminatus* (Coleoptera: Coccinellidae) and its potential as a biological control agent of *Melanaphis sacchari* (Hemiptera: Aphididae). *Ann. Entomol. Soc. Am.* 102, 96–103.
- Akey, D.H., Butler Jr., G.D., 1989. Developmental rates and fecundity of apterous *Aphis gossypii* on seedlings of *Gossypium hirsutum*. *Southwestern Entomol.* 14, 295–299.
- Auad, A.M., Fonseca, M.G., Monteiro, P.H., Resende, T.T., Santos, D.R., 2013. Aspects of the biology of the lady beetle *Diomus seminulus* (Coleoptera: Coccinellidae): a potential biocontrol agent against the yellow sugarcane aphid in Brazil. *Ann. Entomol. Soc. Am.* 106, 243–248.
- Birch, L.C., 1948. The intrinsic rate of natural increase of an insect population. *J. Anim. Ecol.* 17, 15–26.
- Bishop, A.L., Blood, P.R.B., 1978. Temporal distribution and abundance of the coccinellid complex as related to aphid populations on cotton in South-East Queensland. *Aust. J. Zool.* 26, 153–158.
- Blackman, R.L., Eastop, V.F., 2000. *Aphids on the World's Crops: An Identification and Information Guide*. John Wiley and Sons, New York.
- Campbell, A., Frazer, B.D., Gilbert, N., Gutierrez, A.P., Mackauer, M., 1974. Temperature requirements of some aphids and their parasites. *J. Appl. Ecol.* 11, 431–438.
- Chong, J.H., Oetting, R.D., Osborne, L.S., 2005. Development of *Diomus australinus* Gordon (Coleoptera: Coccinellidae) on two mealybug prey species at five constant temperatures. *Biol. Control* 33, 39–48.
- Conway, H.E., Kring, T.J., 2010. Coccinellids associated with the cotton aphid (Homoptera: Aphididae) in northeast Arkansas cotton. *J. Entomol. Sci.* 45, 129–139.
- Dixon, A.F.G., Honek, A., Keil, P., Kotela, M.A.A., Sizling, A.L., Jarosik, V., 2009. Relationship between the minimum and maximum temperature thresholds for development in insects. *Funct. Ecol.* 23, 257–264.
- Enkegaard, A., Brodsgaard, H.F., Hansen, D.L., 2001. *Macrolophus caliginosus*: functional response to whiteflies and preference and switching capacity between whiteflies and spider mites. *Entomol. Exp. Appl.* 101, 81–88.
- Eubanks, M.D., Denno, R.F., 2000. Health food versus fast food: the effects of prey quality and mobility on prey selection by a generalist predator and indirect interactions among prey species. *Ecol. Entomol.* 25, 140–146.
- Furlong, M.J., Zalucki, M.P., 2010. Exploiting predators for pest management: the need for sound ecological assessment. *Entomol. Exp. Appl.* 135, 225–236.
- Hall, D.G., 2001. Notes on the yellow sugarcane aphid *Sipha flava* (Homoptera: Aphididae) and the lady beetle *Diomus terminatus* (Coleoptera: Coccinellidae) in Florida. *J. Am. Soc. Sugarcane Technol.* 21, 21–29.
- Hallborg, K.M., 2003. *Biology and Rearing of Diomus terminatus* (Coleoptera: Coccinellidae) on *Rhopalosiphum maidis* (Homoptera: Aphididae) and Artificial Diet. The University of Florida, Gainesville, 48.
- Henderson, C.F., Tilton, E.W., 1955. Tests with acaricides against the brown wheat mite. *J. Econ. Entomol.* 48, 157–161.
- Hentz, M.G., Nuessly, G.S., 2002. Morphology and biology of *Diomus terminatus* (Coleoptera: Coccinellidae), a predator of *Sipha flava* (Homoptera: Aphididae). *Florida Entomol.* 85, 276–278.
- Herron, G.A., Powis, K., Rophail, J., 2001. Insecticide resistance in *Aphis gossypii* Glover (Hemiptera: Aphididae), a serious threat to Australian cotton. *Aust. J. Entomol.* 40, 85–91.
- Herron, G.A., Wilson, L.J., 2011. Neonicotinoid resistance in *Aphis gossypii* Glover (Aphididae: Hemiptera) from Australian cotton. *Aust. J. Entomol.* 50, 93–98.
- Hillocks, R.J., 2012. Farming with fewer pesticides: EU pesticide review and resulting challenges for UK agriculture. *Crop Prot.* 31, 85–93.
- Hodek, I., 1996. Food relationships. In: Hodek, I., Honek, A. (Eds.), *Ecology of Coccinellidae*. Kluwer, Dordrecht, pp. 143–238.
- Honek, A., 1996. Life history and development. In: Hodek, I., Honek, A. (Eds.), *Ecology of Coccinellidae*. Kluwer, Dordrecht, pp. 61–93.
- Jaworski, C.C., Bompard, A., Genies, L., Amiens-Desneux, E., Desneux, N., 2013. Preference and prey switching in a generalist predator attacking local and invasive alien pests. *PLoS One* 8.
- Johnson, M.-L., Pearce, S., Wade, M., Davies, A., Silberbauer, L., Gregg, P., Zalucki, M., 2000. Review of Beneficials in Cotton Farming Systems. Cotton Research and Development Corporation, Narrabri.
- Kersting, U., Satar, S., Uygun, N., 1999. Effect of temperature on development rate and fecundity of apterous *Aphis gossypii* Glover (Hom., Aphididae) reared on *Gossypium hirsutum* L. *J. Appl. Entomol.* 123, 23–27.
- Manly, B.F.J., 1974. A model for certain types of selection experiments. *Biometrics* 30, 281–294.
- Mensah, R., Leach, D., Young, A., Watts, N., Glennie, P., 2015. Development of *Clitoria ternatea* as a biopesticide for cotton pest management: assessment of product effect on *Helicoverpa* spp. and their natural enemies. *Entomol. Exp. Appl.* 154, 131–145.
- Meyerdirk, D.E., 1983. Biology of *Diomus flavifrons* (Blackburn) (Coleoptera: Coccinellidae), a citrus mealybug predator. *Environ. Entomol.* 12, 1275–1277.
- Meyling, N.V., Enkegaard, A., Brodsgaard, H., 2003. Two Anthocoris bugs as predators of glasshouse aphids – voracity and prey preference. *Entomol. Exp. Appl.* 108, 59–70.
- Pang, H., Slipinski, A., 2009. Revision of the Australian Coccinellidae (Coleoptera). Genus *Diomus* Mulsant. Part 1. *Ann. Zool.* 59, 641–698.
- Parajulee, M.N., 2007. Influence of constant temperatures on life history parameters of the cotton aphid, *Aphis gossypii*, infesting cotton. *Environ. Entomol.* 36, 666–672.
- Pyke, B., McIntyre, G., 2007. The role and achievements of a national cotton extension program. In: World Cotton Research Conference–4, Lubbock, Texas, USA, 10–14 September 2007 (unpaginated).
- Rosenheim, J.A., Willhoit, L.R., Colfer, R.G., 1994. Seasonal biology and polymorphism of the cotton aphid, *Aphis gossypii*, in California. In: Proceedings of the Beltwide Cotton Conference, National Cotton Council, January 5–8, San Diego, California, pp. 125–131.
- Shrestha, G., Enkegaard, A., 2013. The green lacewing, *Chrysoperla carnea*: preference between lettuce aphids, *Nasonovia ribisnigri*, and western flower thrips, *Frankliniella occidentalis*. *J. Insect Sci.* 13, 1–10.
- Sokal, R.R., Rohlf, F.J., 1995. *Biometry*. W.H. Freeman and Company, San Francisco, CA.
- Stanley, J.N., 1997. The Seasonal Abundance and Impact of Predatory Arthropods on *Helicoverpa* Species in Australian Cotton Fields. University of New England, Armidale, pp. 282.
- Straub, C.S., Snyder, W.E., 2006. Species identity dominates the relationship between predator biodiversity and herbivore suppression. *Ecology* 87, 277–282.
- Tift, K.H., Leppla, N.C., Osborne, L.S., Cuda, J.P., 2006. Rearing *Diomus terminatus* (Coleoptera: Coccinellidae) on the corn leaf aphid, *Rhopalosiphum maidis* (Homoptera: Aphididae). *Florida Entomol.* 89, 263–265.
- Williams, S., Wilson, L.J., Vogel, S., 2011. Pests and Beneficials in Australian Cotton Landscapes. Cotton Catchment Communities CRC, Narrabri.
- Xia, J.Y., van der Werf, W., Rabbinge, R., 1999. Influence of temperature on bionomics of cotton aphid, *Aphis gossypii*, on cotton. *Entomol. Exp. Appl.* 90, 25–35.
- Yu, L., Chen, Z., Zheng, F., Shi, A., Guo, T., Yeh, B., Chi, H., Xu, Y., 2013. Demographic analysis, a comparison of the jackknife and bootstrap methods, and predation projection: a case study of *Chrysopa pallens* (Neuroptera: Chrysopidae). *J. Econ. Entomol.* 106, 1–9.
- Zalucki, M.P., Adamson, D., Furlong, M.J., 2009. The future of IPM: whither or wither? *Aust. J. Entomol.* 48, 85–96.
- Zalucki, M.P., Furlong, M.J., Schellhorn, N.A., Macfadyen, S., Davies, A.P., 2015. Assessing the impact of natural enemies in agroecosystems: toward “real” IPM or in quest of the Holy Grail? *Insect Sci.* 22, 1–5.